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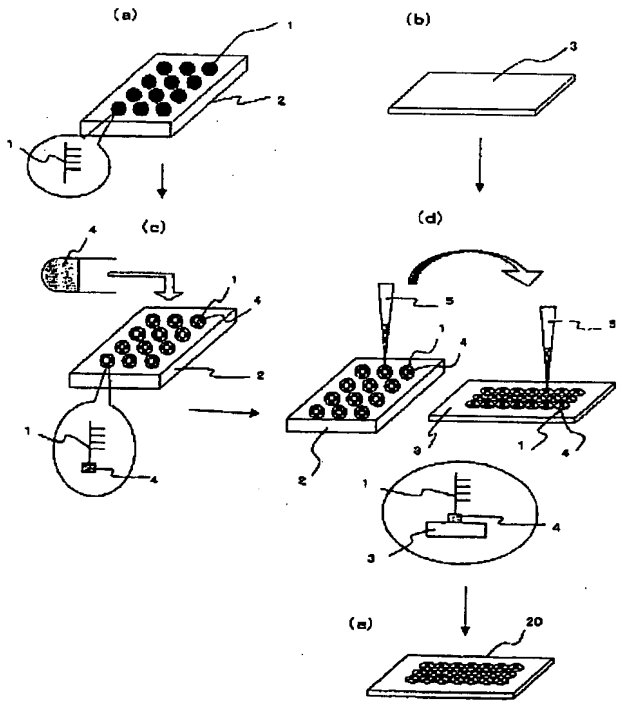
(54)【発明の名称】　バイオチップ及びその製造方法

(57)【要約】
【課題】　プレートのプローブをプロットした以外の部分にサンプルＤＮＡが付着することのないバイオチップを提供する。
【解決手段】　プローブ１と結合剤４とを混合した混合物をプレート３に植え付ける。あるいは、プローブを植え付けるべきプレート上の位置に、先ずプローブとプレートとの結合剤を局所的に付着させ、次に、結合剤が付着しているプレート上の位置にプローブを植え付ける。こうして、プローブをプロットしたか所為が異の箇所に結合剤が付着していないバイオチップ２０を製造する。



【特許請求の範囲】

【請求項１】　プレート上の複数の位置にプレートとプローブとを結合させる結合剤を用いてプローブを植え付けたバイオチップにおいて、前記結合剤は前記プローブが植え付けられている位置に局在していることを特徴とするバイオチップ。

【請求項２】　プレートに該プレートとプローブを結合させる結合剤を用いてプローブを植え付けてバイオチップを製造するバイオチップの製造方法において、
プローブと結合剤とを混合した混合物をプレートに植え付けることを特徴とするバイオチップの製造方法。

【請求項３】　プレートにプローブを植え付けてバイオチップを製造するバイオチップの製造方法において、
プローブを植え付けるべきプレート上の位置に、前記プローブと前記プレートとの結合剤を局所的に付着させるステップと、
前記結合剤が付着しているプレート上の位置にプローブを植え付けるステップとを含むことを特徴とするバイオチップの製造方法。

【請求項４】　請求項２又は３記載のバイオチップの製造方法において、前記プレートはガラス製であることを特徴とするバイオチップの製造方法。

【請求項５】　請求項２～４のいずれか１項記載のバイオチップの製造方法において、ピンヘッドが窪んだピンを用いてプローブを植え付けることを特徴とするバイオチップの製造方法。

【請求項６】　プレートにプローブを植え付けるのに使用されるピンにおいて、プローブを付着させるピンヘッドが窪んでいることを特徴とするピン。

【請求項７】　プレートにプローブを植え付けるのに使用されるピンにおいて、プローブを付着させるピンヘッドに十字型の溝を形成したことを特徴とするピン。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、複数種類のプローブをプレートにスポットしたバイオチップに関する。

【０００２】

【従来の技術】従来から、複数種類のＤＮＡ、ＲＮＡ、たんぱく質等の生体高分子からなるプローブをガラスなどのプレートにスポットして、バイオチップを製造することが行われていた。図４は、この従来の方法の原理を説明する図である。図４（ａ）に示すように、複数種類のプローブＤＮＡ１が入っているマイクロプレート２を用意する。一方、図４（ｂ）に示すよう、プレート３としてガラス板を用意しておき、図４（ｃ）で示すように、プレート３の表面にpoly-l-LysineをＤＮＡとガラスの結合剤４としてコーティングする。この後、図４（ｄ）で示すように、マイクロプレート２に入っているプローブＤＮＡ１をピンに付着させ、表面にＤＮＡとガラスの結合剤（poly-l-Lysine）４がコーテイングしてあるガラスプレート３の上に、ピン５に付着させたプローブＤＮＡ１を接触させてスポットする。マイクロプレート２に入っている全てのプローブＤＮＡをスポットし終わるまでこの作業を繰り返し、図４（ｅ）に示すバイチップを製造していた。このように、従来はプレートに予めＤＮＡとガラスの結合剤を全面コーティングし、その上にＤＮＡをプロットしてバイオチップを製造していた。

【０００３】図５は、バイチップを利用したハイブリダイゼーションの原理を説明する図である。図５（ａ）に示すように、プローブＤＮＡ１が結合剤４でガラスのプレート３にスポットされているバイチップと、蛍光物質１０で標識したサンプルＤＮＡ１１を、ともにハイブリダイゼーション溶液に入れてハイブリダイズさせる。ハイブリダイゼーション溶液は、ホルムアルデヒド、ＳＳＣ（NaCl, trisodiumcitrate）、ＳＤＳ（sodium dodecyl sulfate）、ＥＤＴＡ（ethylenediamidetetraacetic acid）、蒸留水などからなる混合液であり、混合比率は使用するＤＮＡの性質により異なる。

【０００４】このとき、サンプルＤＮＡ１１とバイチップ上のプローブＤＮＡ１が相補鎖ＤＮＡであれば、両者は二重らせん構造をとり結合する。一方、両者が相補鎖でなければ結合せず、蛍光物質１０で標識したサンプルＤＮＡ１１がガラスのプレート３上にコーティングされている結合剤４と結合し、ガーベージとして残る。

【０００５】その後、図５（ｂ）に示すように、ガラスのプレート３上に残った蛍光物質１０で標識したサンプルＤＮＡ１１を水１２の中に入れて洗い流すと、プローブＤＮＡ１と結合していないサンプルＤＮＡ１１は排出される。その後、図５（ｃ）に示すように、プローブＤＮＡと結合しているサンプルＤＮＡに標識している蛍光物質をランプ１４からの光エネルギーで励起させ、蛍光物質が励起して発光する光をＣＣＤなどの光センサー１３で検出することでハイブリダイゼーションの検出を行う。

【０００６】

【発明が解決しようとする課題】バイチップを用いた実験では、バイチップにサンプルＤＮＡをふりかけ、バイチップにスポットしてあるプローブＤＮＡとハイブリダイズさせ、どのプローブＤＮＡとサンプルＤＮＡが結合したかを検出している。検出の前処理として、ハイブリダイズしたあとに、結合しなかったサンプルＤＮＡを排除するためにバイオチップを水で洗っている。しかし、プレートにＤＮＡとガラスの結合剤を全面コーティングしているため、プローブＤＮＡと結合しなかったサンプルＤＮＡが必要以外の部分、すなわち、プローブＤＮＡがスポットされていない結合剤部分に張り付き、結合剤４と結合しているサンプルＤＮＡ１１は水洗いによってもガラスのプレート３上から除去されない。それが検出時ノイズとなって現われ、感度が低くなっていた。つま

り、サンプルDNAの一部がプローブDNAとの特異結合ではなく単に結合剤4に張り付いた状態でバイオチップ上にガーベージとして残り、そのサンプルDNAに標識されている蛍光物質も励起されて発光するため、ノイズとして検出され、S／Nが悪くなるという問題があった。

【0007】本発明は、このような従来技術の問題点に鑑みなされたもので、プレートのプローブをプロットした以外の部分にサンプルDNAが付着することのないバイオチップを提供すること、及びそのバイオチップの製造方法を提供することを目的とする。

【0008】

【課題を解決するための手段】前記目的を達成するために、本発明では、プローブをスポットするプレート上の特定部分にだけプローブとガラスの結合剤を付着させる。すなわち、プレートにプローブをスポットした部分以外にはプローブとガラスの結合剤を付着させないため、サンプルDNAを入れてハイブリダイズさせた後、プローブと結合しなかったサンプルDNAは水で洗い流せばチップ上から無くなるため、検出時ノイズが無くなりS／Nのを向上させることができ、高感度となる。

【0009】すなわち、本発明によるバイオチップは、プレート上の複数の位置にプレートとプローブとを結合させる結合剤を用いてプローブを植え付けたバイオチップにおいて、結合剤はプローブが植え付けられている位置に局在していることを特徴とする。

【0010】本発明によるバイオチップの製造方法は、プレートに該プレートとプローブを結合させる結合剤を用いてプローブを植え付けてバイオチップを製造するバイオチップの製造方法において、プローブと結合剤とを混合した混合物をプレートに植え付けることを特徴とする。

【0011】本発明によるバイオチップの製造方法は、また、プレートにプローブを植え付けてバイオチップを製造するバイオチップの製造方法において、プローブを植え付けるべきプレート上の位置に、プローブとプレートとの結合剤を局所的に付着させるステップと、結合剤が付着しているプレート上の位置にプローブを植え付けるステップとを含むことを特徴とする。プレートはガラス製とすることができる。また、ピンヘッドが窪んだピンを用いてプローブを植え付けるのが好ましい。

【0012】本発明のピンは、プレートにプローブを植え付けるのに使用されるピンにおいて、プローブを付着させるピンヘッドが窪んでいることを特徴とする。本発明のピンは、また、プレートにプローブを植え付けるのに使用されるピンにおいて、プローブを付着させるピンヘッドに十字型の溝を形成したことを特徴とする。

【0013】

【発明の実施の形態】以下、本発明の実施形態について説明する。ここでは、プローブとしてDNAを用いる場

合について説明する。しかし、プローブとして用いることができるのはDNAに限られず、RNAあるいはたんぱく質をプローブとして用いることもできる。また、プレートとしてガラスプレートを用いた例で説明するが、ガラス以外にナイロン製のメンブレン等も使用可能である。

【0014】図1は、本発明の第1の実施の形態の原理を説明する図である。図1（a）に示すように、マイクロプレート2には複数種類のプローブDNA1が入っている。図1（b）に示すように、バイオチップのプレートとしてはガラスのプレート3を使用する。図1（c）に示すように、DNAとガラスとの結合剤4をマイクロプレートの各ウェルに分注し、プローブDNA1と混合する。DNAとガラスとの結合剤4としては、例えばpoly-l-Lysineあるいはカルボジイミドを用いることができる。

【0015】次に、図1（d）に示すように、結合剤4とプローブDNA1を混ぜたものをピン5で吸い上げ（もしくは溶液をピン5の先に付着させて）、プレート3にスポットする。この処理をマイクロプレート2に入っているすべてのプローブDNAに対して繰り返し行うことで、図1（e）に示すように、必要な部分にのみ結合剤4が付着し、プローブをスポットした以外の部分には結合剤4が付着していないバイチップ20を作ることができる。

【0016】図2は、本発明の第2の実施の形態の原理を説明する図である。図2（a）に示すように、マイクロプレート2にはプローブDNA1が入っている。図2（b）に示すように、バイチップのプレートとしてガラスプレート3を使用する。図2（c）に示すように、結合剤4を例えば毛細管6で吸い上げ、ガラスプレート3上のプローブDNAをスポットする位置に吐き出す。こうすることで、ガラスプレート3上のプローブDNA1をスポットする位置に、予め結合剤4をスポットして付着させておく。その後、図2（d）に示すように、マイクロプレート2に入っているプローブDNA1を結合剤4でフォーマットされたプレート3に、ピン5で吸い上げ（もしくはピン5に溶液を付着させて）繰り返しスポットする。こうして、図2（e）に示すように、必要な部分にのみ結合剤4が付着し、プローブをスポットした以外の部分には結合剤4が付着していないバイチップ20を作ることができる。

【0017】図6に、本発明で用いたピン5のヘッド形状を示す。図6（a）に示したピン5aは、ヘッド部分、すなわちプローブを付着させる部分がアーチ型に窪んだピンである。また、図6（b）に示したピン5bは、ヘッド部分がアーチ型に窪み、さらに窪みの中に十字型に溝を切ったピンである。ピンのヘッドをアーチ状に窪ませることで、スポットするプローブの中にピンを入れたときに表面張力でうまくピンヘッドにプローブ溶

液が吸い込まれるしくみになっている。アーチの深さは任意である。このピンを使うことで、ヘッドが平坦な従来のピンを用いる場合より、プレートへのＤＮＡスポット量を約１０倍に増やすことができる。図６（ｃ）に示したピン５ｃは、平坦なヘッドの表面に十字型に溝を切ったものである。このピンによってもヘッドが単に平坦な従来のピンに比較してスポット量を増やすことができる。

【００１８】図３は、本発明によるバイチップを利用したハイブリダイゼーションの原理を説明した図である。図３（ａ）に示すように、プローブＤＮＡ１が結合剤４でガラスプレート３にスポットされているバイチップ２０と、蛍光物質１０で標識したサンプルＤＮＡ１１を、ともにハイブリダイゼーション溶液に入れてハイブリダイズさせる。ハイブリダイゼーション溶液は、ホルムアルデヒド、ＳＳＣ（NaCl, trisodium citrate）、ＳＤＳ（sodium dodecyl sulfate）、ＥＤＴＡ（ethylenediamidetetraacetic acid）、蒸留水などからなる混合液であり、混合比率は使用するＤＮＡの性質により異なる。

【００１９】このとき、サンプルＤＮＡ１１とバイチップ上のプローブＤＮＡ１が相補鎖ＤＮＡであれば、両者は二重らせん構造をとり結合する。一方、両者が相補鎖でなければ結合せず、蛍光物質１０で標識したサンプルＤＮＡ１１がガラスのプレート３上にガーベージとして残る。図３（ｂ）に示すように、ガラスのプレート３上に残った蛍光物質１０で標識したサンプルＤＮＡ１１を水１２の中に入れて洗い流すと、ガラスとＤＮＡは結合が弱いため、ガーベージのサンプルＤＮＡ１１が排出され、ガラスのプレート３上から無くなる。図３（ｃ）に

示すように、ハイブリダイゼーションの検出は結合しているサンプルＤＮＡに標識している蛍光物質をランプ１４の光で励起させ、蛍光物質が励起して発光する光をＣＣＤ等の光センサー１３で検出する。このとき、バイオチップ２０上にガーベージのサンプルＤＮＡが無いため、検出のＳ／Ｎ比が向上する。

【００２０】

【発明の効果】本発明によると、プローブをスポットする部分のみに結合剤を付着させたバイオチップを製造することができ、バイチップ読取り時、検出感度を向上させることができる。

【図面の簡単な説明】

【図１】本発明によるバイオチップの製造方法の一例を説明する図。

【図２】本発明によるバイオチップの製造方法の他の例を説明する図。

【図３】本発明のバイチップを用いたハイブリダイゼーションと検出の説明図。

【図４】従来のバイオチップの製造方法を説明する図。

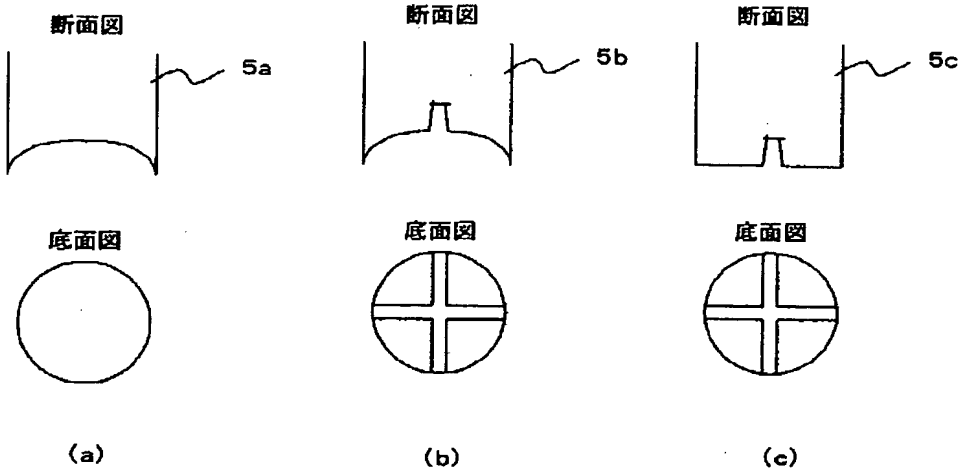
【図５】従来のバイチップを用いたハイブリダイゼーションと検出の説明図。

【図６】本発明によるプローブ付着用のピンの説明図。

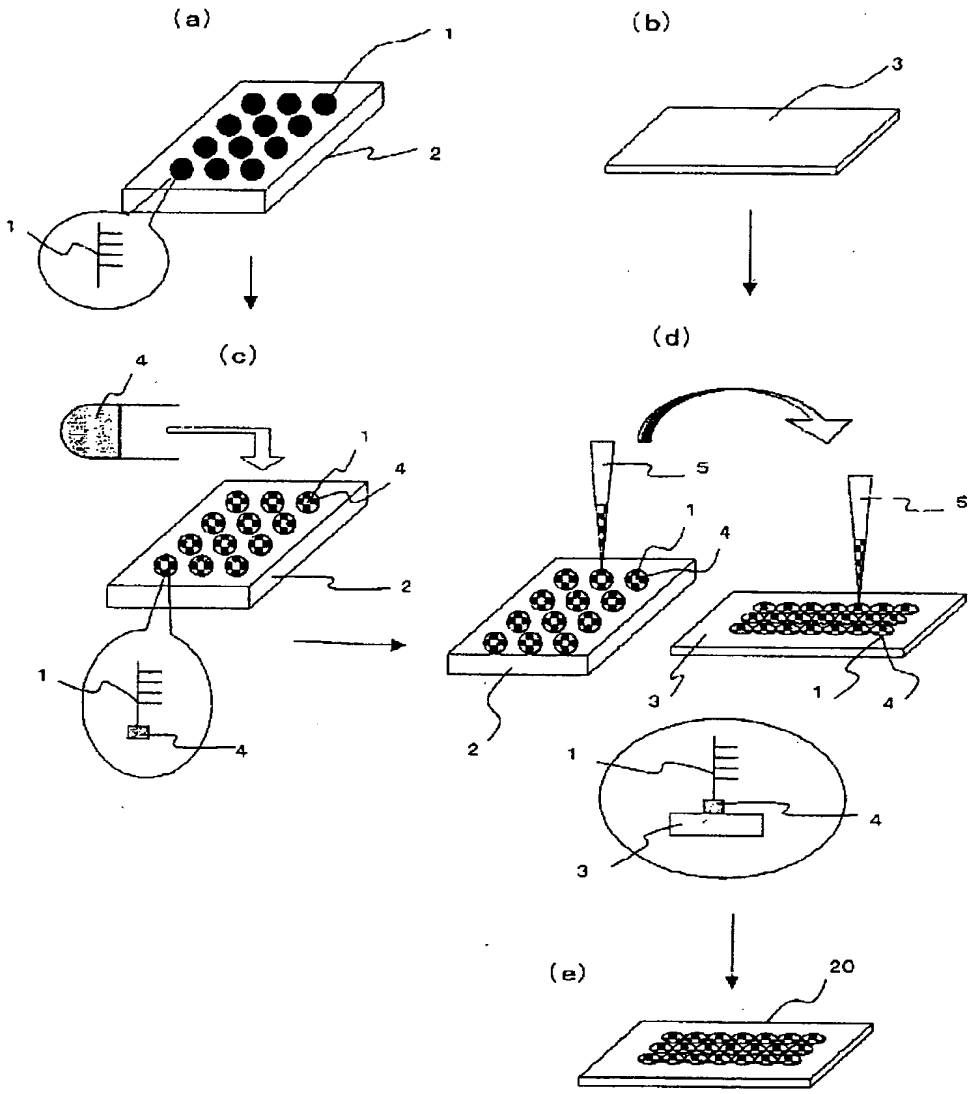
【符号の説明】

１…プローブＤＮＡ、２…プローブ格納用マイクロプレート、３…ガラスのプレート、４…結合剤、５…ピン、６…毛細管、１０…蛍光物質、１１…サンプルＤＮＡ、１２…水、１３…光センサー、１４…ランプ、２０…バイオチップ。

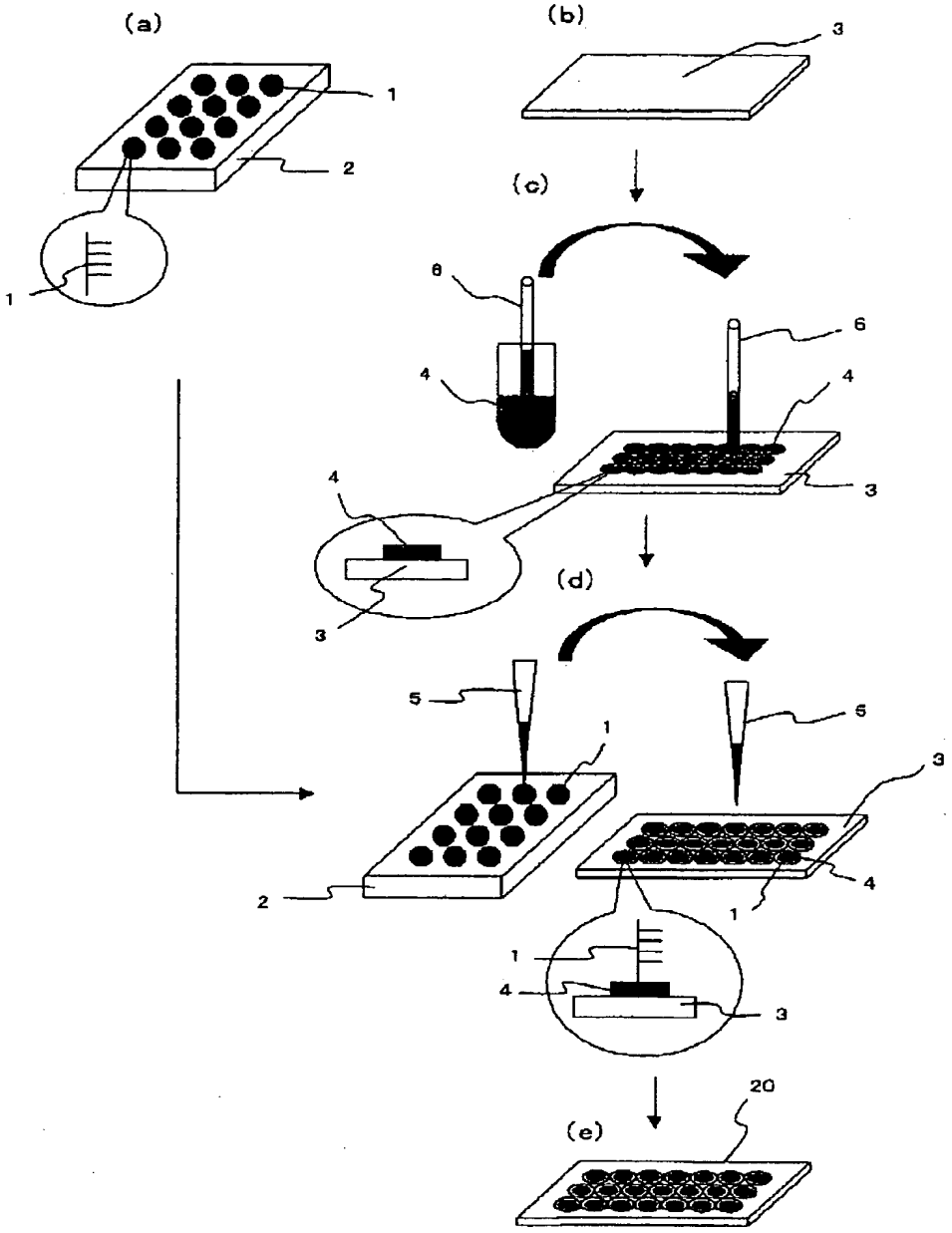
【図６】



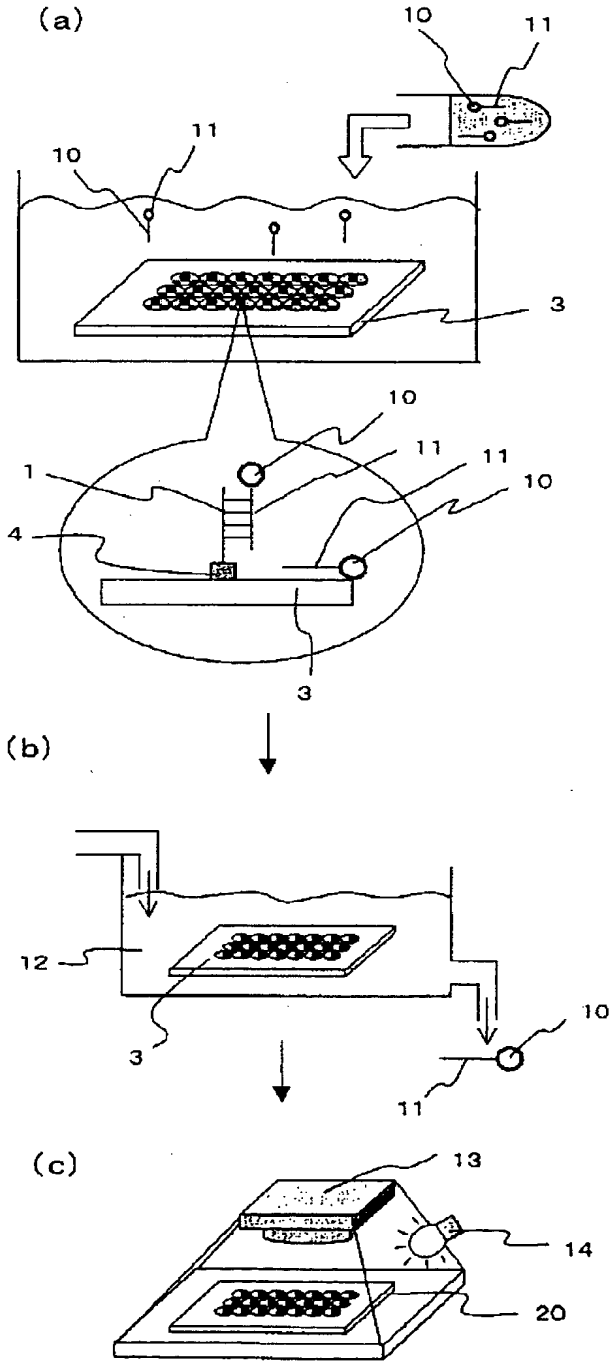
【図１】



【図２】



【図3】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(b)
12
3
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(c)
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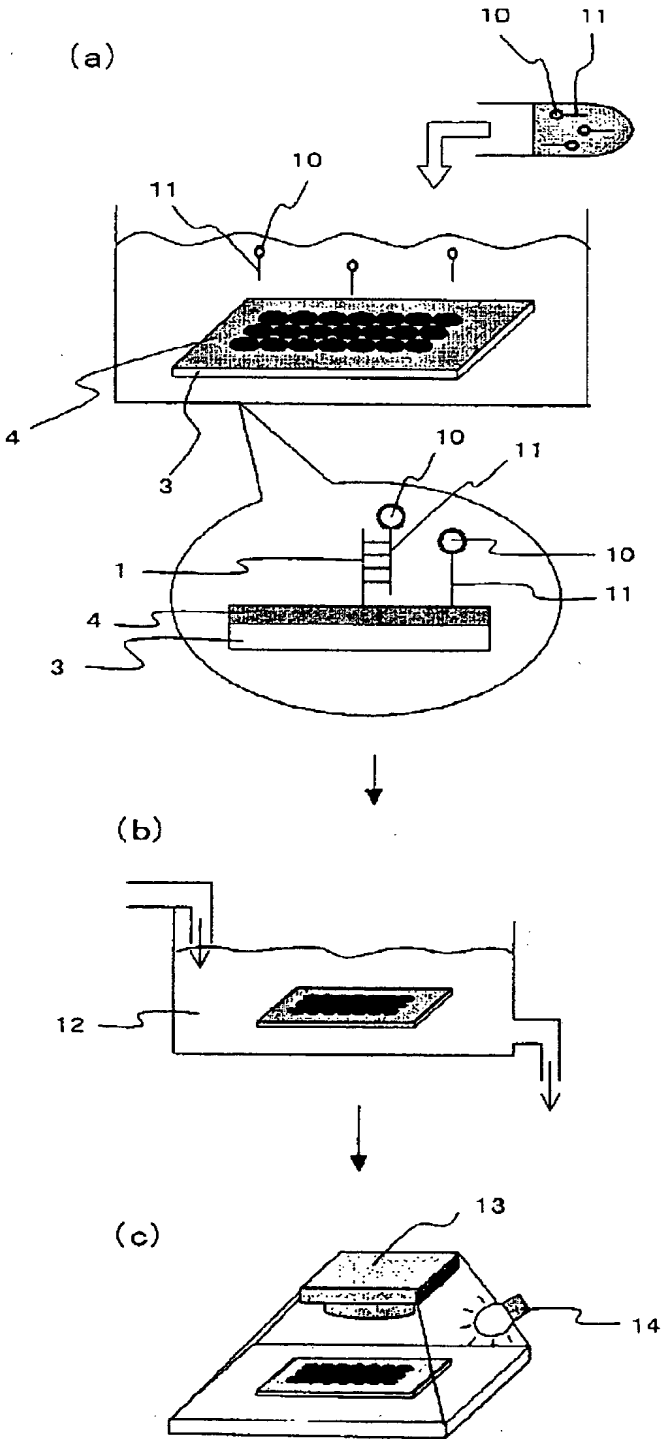
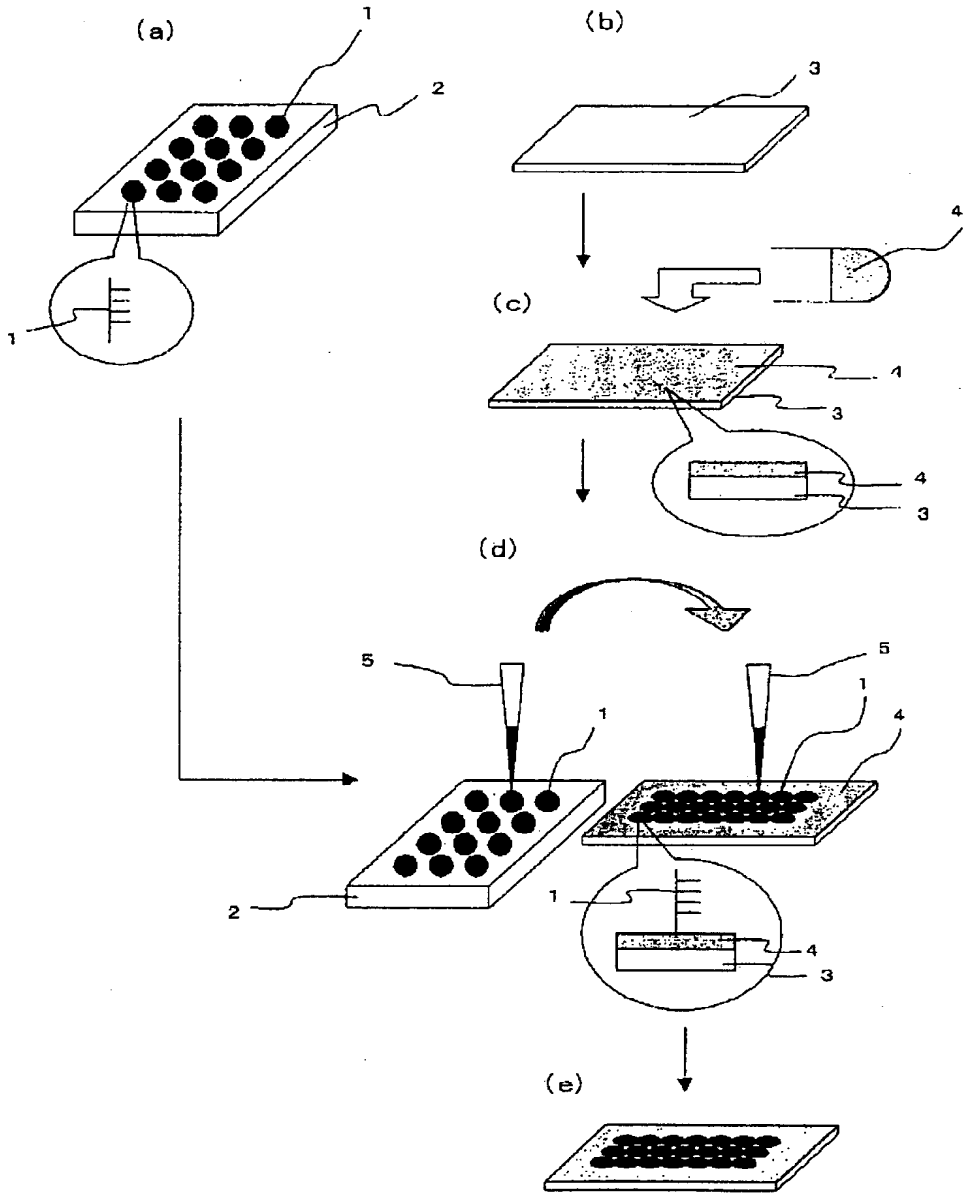
20

【図5】


(a)
10
11
11
10
4
3
10
11
1
10
11
4
3
(b)
12
(c)
13
14

【図４】



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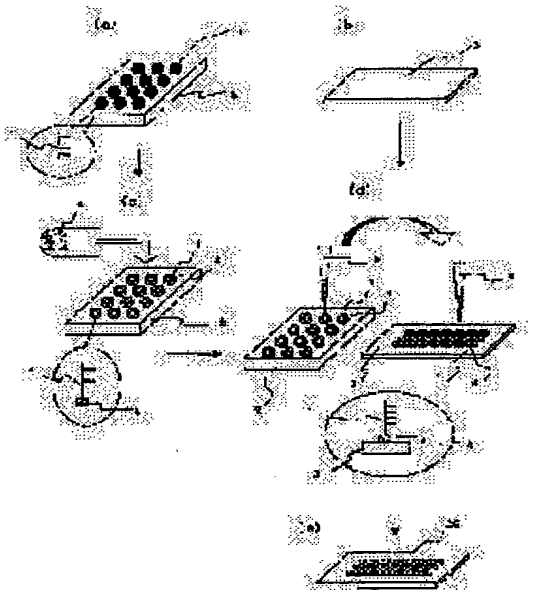
(54) BIOCHIP AND ITS PRODUCTION

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a biochip not allowing a sample DNA to adhere to the parts other than the part where the probe of a plate is plotted.
SOLUTION: A mixture obtained by mixing a probe 1 with a binder 4 is inoculated to a plate 3, or the binder of the probe and the plate is topically attached to the position on the plate to be inoculated at first, and the probe is inoculated to a position on the plate to which the binder adheres to produce the objective biochip 20 having no stuck binder in a part other than the parts where the probe is plotted.



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CLAIMS

[Claim(s)]

[Claim 1] The aforementioned binder is a biochip characterized by carrying out localization to the position in which the aforementioned probe is planted in the biochip which planted the probe in two or more positions on a plate using the binder which combines a plate and a probe.

[Claim 2] The manufacture method of the biochip characterized by planting in a plate the mixture which mixed the probe and the binder in the manufacture method of the biochip which plants a probe in a plate using the binder which combines this plate and a probe, and manufactures a biochip.

[Claim 3] The manufacture method of the biochip characterized by including the step which makes the binder of the aforementioned probe and the aforementioned plate adhere to the position on the plate in which a probe should be planted locally, and the step which plants a probe in the position on the plate to which the aforementioned binder has adhered in the manufacture method of the biochip which plants a probe in a plate and manufactures a biochip.

[Claim 4] It is the manufacture method of the biochip characterized by the aforementioned plate being glass in the manufacture method of a biochip according to claim 2 or 3.

[Claim 5] The manufacture method of the biochip characterized by planting a probe using the pin by which the pin head became depressed in the manufacture method of the biochip of a claim 2–4 given in any 1 term.

[Claim 6] The pin characterized by the pin head to which a probe is made to adhere having become depressed in the pin used for planting a probe in a plate.

[Claim 7] The pin characterized by forming a cross-joint type slot in the pin head to which a probe is made to adhere in the pin used for planting a probe in a plate.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to the biochip which carried out the spot of two or more kinds of probes to the plate.
[0002]
[Description of the Prior Art] Carrying out the spot of the probe which consists of biopolymers, such as two or more kinds of DNA, RNA, and protein, to plates, such as glass, and manufacturing a biochip from the former, was performed. Drawing 4 is drawing explaining the principle of this conventional method. As shown in drawing 4 (a), the microplate 2 containing two or more kinds of probe DNA 1 is prepared. On the other hand, as shown in drawing 4 (b), the glass plate is prepared as a plate 3, and as drawing 4 (c) shows, the front face of a plate 3 is coated with poly-l-Lysine as a binder 4 of DNA and glass. Then, as drawing 4 (d) shows, the probe DNA 1 included in the microplate 2 is made to adhere to a pin, DNA and the probe DNA 1 made to adhere to a pin 5 on the glass plate 3 with which the binder (poly-l-Lysine) 4 of glass is coated are contacted on a front face, and a spot is carried out to it. This work was repeated until it finished carrying out the spot of all the probe DNA included in the microplate 2, and BAICHIPPU shown in drawing 4 (e) was manufactured. Thus, conventionally, the plate was beforehand coated with the binder of DNA and glass completely, on it, DNA was plotted and the biochip was manufactured.
[0003] Drawing 5 is drawing explaining the principle of the hybridization using BAICHIPPU. Probe DNA 1 makes both hybridization solutions put in and hybridize the sample DNA 11 which carried out the indicator to BAICHIPPU by which the spot is carried out to the plate 3 of glass with the fluorescent substance 10 by the binder 4, as shown in drawing 5 (a). A hybridization solution is mixed liquor which consists of formaldehyde, SSC (NaCl, trisodiumcitrate) and SDS (sodium dodecyl sulfate), EDTA (ethylenediamidetetraacetic acid), distilled water, etc., and a mixed ratio changes with properties of DNA to be used.
[0004] If the probe DNA 1 on a sample DNA 11 and BAICHIPPU is a complementary strand DNA at this time, both will take a double helix structure and will join together. On the other hand, if both are not complementary strands, it will not join together, but the sample DNA 11 which carried out the indicator with the fluorescent substance 10 combines with the binder 4 coated on the plate 3 of glass, and remains as garbage.
[0005] Then, if the sample DNA 11 which carried out the indicator is put in into water 12 and flushed with the fluorescent substance 10 which remained on the plate 3 of glass as shown in drawing 5 (b), the sample DNA 11 which has not been combined with probe DNA 1 will be discharged. Then, as shown in drawing 5 (c), hybridization is detected by detecting the light which the fluorescent substance which is carrying out the indicator to the sample DNA combined with probe DNA is excited by the light energy from a lamp 14, and a fluorescent substance excites, and emits light by the photosensors 13, such as CCD.
[0006]
[Problem(s) to be Solved by the Invention] In the experiment using BAICHIPPU, sprinkled Sample DNA over BAICHIPPU, and it was made to hybridize with the probe DNA which has carried out the spot to BAICHIPPU, and has detected which probe DNA and Sample DNA joined together. As pretreatment of detection, after hybridizing, in order to eliminate the sample DNA which was not combined, the biochip is washed with water. However, since the plate is completely coated with the binder of DNA and glass, the sample DNA which was not combined with probe DNA sticks to portions other than the need, i.e., the binder portion to which the spot of the probe DNA is not carried out, and the sample DNA 11 combined with the binder 4 is not removed from on the plate 3 of glass by washing in cold water, either. It became a noise at the time of detection, and

appeared, and sensitivity was low. That is, since it remained as garbage on a biochip not in the unique combination with probe DNA but in the state where it only stuck to the binder 4, the fluorescent substance by which the indicator is carried out to the sample DNA was also excited and a part of sample DNA emitted light, it was detected as a noise and there was a problem that S/N became bad.

[0007] this invention was made in view of the trouble of such conventional technology, and aims at offering the biochip in which Sample DNA does not adhere to the portion except having plotted the probe of a plate, and offering the manufacture method of the biochip.

[0008]

[Means for Solving the Problem] In order to attain the aforementioned purpose, the binder of a probe and glass is made to adhere only to the particular part on the plate which carries out the spot of the probe in this invention. That is, in order not to make the binder of a probe and glass adhere in addition to the portion which carried out the spot of the probe to the plate, after making Sample DNA put in and hybridize, since the sample DNA which was not combined with a probe will disappear from on a chip if it washes away with water, the noise of it can be lost at the time of detection, it can raise S/N's, and serves as high sensitivity.

[0009] That is, in the biochip by which the biochip by this invention planted the probe in two or more positions on a plate using the binder which combines a plate and a probe, it is characterized by carrying out localization of the binder to the position in which the probe is planted.

[0010] The manufacture method of the biochip by this invention is characterized by planting in a plate the mixture which mixed the probe and the binder in the manufacture method of the biochip which plants a probe in a plate using the binder which combines this plate and a probe, and manufactures a biochip.

[0011] The manufacture method of the biochip by this invention is characterized by including the step which makes the binder of a probe and a plate adhere to the position on the plate in which a probe should be planted locally, and the step which plants a probe in the position on the plate to which the binder has adhered in the manufacture method of the biochip which plants a probe in a plate and manufactures a biochip again. A plate can be made glass. Moreover, it is desirable to plant a probe using the pin by which the pin head became depressed.

[0012] The pin of this invention is characterized by the pin head to which a probe is made to adhere having become depressed in the pin used for planting a probe in a plate. The pin of this invention is characterized by forming a cross-joint type slot in the pin head to which a probe is made to adhere in the pin used for planting a probe in a plate again.

[0013]

[Embodiments of the Invention] Hereafter, the operation gestalt of this invention is explained. Here, the case where DNA is used as a probe is explained. However, only DNA can be used as a probe and it can also use RNA or protein as a probe. Moreover, although the example using the glass plate as a plate explains, the membrane made of nylon etc. is usable in addition to glass.

[0014] Drawing 1 is drawing explaining the principle of the gestalt of operation of the 1st of this invention. As shown in drawing 1 (a), two or more kinds of probe DNA 1 is contained in the microplate 2. As shown in drawing 1 (b), the plate 3 of glass is used as a plate of a biochip. As shown in drawing 1 (c), the binder 4 of DNA and glass is poured distributively to each well of a microplate, and it mixes with probe DNA 1. As a binder 4 of DNA and glass, poly-l-Lysine or a carbodiimide can be used, for example.

[0015] next, what mixed probe DNA 1 with the binder 4 as shown in drawing 1 (d) is sucked up by the pin 5 (or a solution is adhered to the point of a pin 5 — making), and a spot is carried out to a plate 3 As shown in drawing 1 (e), BAICHIPPU 20 in which a binder 4 adheres only to a required portion and the binder 4 has not adhered to the portion except having carried out the spot of the probe can be made from performing this processing repeatedly to all the probe DNA included in the microplate 2.

[0016] Drawing 2 is drawing explaining the principle of the gestalt of operation of the 2nd of this invention. As shown in drawing 2 (a), probe DNA 1 is contained in the microplate 2. As shown in drawing 2 (b), the glass plate 3 is used as a plate of BAICHIPPU. As shown in drawing 2 (c), a binder 4 is sucked up with a vas capillare 6, and it breathes out in the position which carries out the spot of the probe DNA on the glass plate 3. The spot of the binder 4 is carried out to the position which carries out the spot of the probe DNA 1 on the glass plate 3, and it is made to adhere to it beforehand by carrying out like this. then, the probe DNA 1 which is contained in the microplate 2 as shown in drawing 2 (d) is sucked up by the pin 5 on the plate 3 formatted by the binder 4 (or a solution is adhered to a pin 5 — making), and a repeat spot is carried out In this way, as shown in drawing 2 (e), BAICHIPPU 20 in which a binder 4 adheres only to a required portion and

the binder 4 has not adhered to the portion except having carried out the spot of the probe can be made.
[0017] The head configuration of the pin 5 used for drawing 6 by this invention is shown. Pin 5a shown in drawing 6 (a) is the pin by which the head portion, i.e., the portion to which a probe is made to adhere, became depressed in the arch type. Moreover, pin 5b shown in drawing 6 (b) is the pin which the head portion became depressed in the arch type, and cut the slot to the cross-joint type in the hollow further. By hollowing the head of a pin in the shape of an arch, when a pin is put in into the probe which carries out a spot, it is the structure from which a probe solution is well sucked in by the pin head with surface tension. The depth of an arch is arbitrary. By using this pin, the amount of DNA spots to a plate can be increased about 10 times from the case where the conventional pin with a flat head is used. Pin 5c shown in drawing 6 (c) cuts a slot to a cross-joint type on the front face of a flat head. A head can increase the amount of spots also by this pin as compared with the only flat conventional pin.
[0018] Drawing 3 is drawing explaining the principle of the hybridization using BAICHIPPU by this invention. Probe DNA 1 makes both hybridization solutions put in and hybridize the sample DNA 11 which carried out the indicator to BAICHIPPU 20 by which the spot is carried out to the glass plate 3 with the fluorescent substance 10 by the binder 4, as shown in drawing 3 (a). A hybridization solution is mixed liquor which consists of formaldehyde, SSC (NaCl, trisodium citrate) and SDS (sodium dodecyl sulfate), EDTA (ethylenediamidetetraacetic acid), distilled water, etc., and a mixed ratio changes with properties of DNA to be used.
[0019] If the probe DNA 1 on a sample DNA 11 and BAICHIPPU is a complementary strand DNA at this time, both will take a double helix structure and will join together. On the other hand, if both are not complementary strands, it will not join together, but the sample DNA 11 which carried out the indicator with the fluorescent substance 10 remains as garbage on the plate 3 of glass. If the sample DNA 11 which carried out the indicator is put in into water 12 and flushed with the fluorescent substance 10 which remained on the plate 3 of glass as shown in drawing 3 (b), since combination is weak, the sample DNA 11 of garbage will be discharged and glass and DNA will disappear from on the plate 3 of glass. As shown in drawing 3 (c), detection of hybridization excites the fluorescent substance which is carrying out the indicator to the united sample DNA with the light of a lamp 14, and detects the light in which a fluorescent substance excites and emits light by the photosensors 13, such as CCD. In order that there may be no sample DNA of garbage on a biochip 20 at this time, the S/N ratio of detection improves.
[0020]
[Effect of the Invention] According to this invention, the biochip which made the binder adhere only to the portion which carries out the spot of the probe can be manufactured, and detection sensitivity can be raised at the time of BAICHIPPU read.

[Translation done.]

TECHNICAL FIELD

[The technical field to which invention belongs] this invention relates to the biochip which carried out the spot of two or more kinds of probes to the plate.

[Translation done.]

PRIOR ART

[Description of the Prior Art] Carrying out the spot of the probe which consists of biopolymers, such as two or more kinds of DNA, RNA, and protein, to plates, such as glass, and manufacturing a biochip from the former, was performed. Drawing 4 is drawing explaining the principle of this conventional method. As shown in drawing 4 (a), the microplate 2 containing two or more kinds of probe DNA 1 is prepared. On the other hand, as shown in drawing 4 (b), the glass plate is prepared as a plate 3, and as drawing 4 (c) shows, the front face of a plate 3 is coated with poly-l-Lysine as a binder 4 of DNA and glass. Then, as drawing 4 (d) shows, the probe DNA 1 included in the microplate 2 is made to adhere to a pin, DNA and the probe DNA 1 made to adhere to a pin 5 on the glass plate 3 with which the binder (poly-l-Lysine) 4 of glass is coated are contacted on a front face, and a spot is carried out to it. This work was repeated until it finished carrying out the spot of all the probe DNA included in the microplate 2, and BAICHIPPU shown in drawing 4 (e) was manufactured. Thus, conventionally, the plate was beforehand coated with the binder of DNA and glass completely, on it, DNA was plotted and the biochip was manufactured.

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[0005] Then, if the sample DNA 11 which carried out the indicator is put in into water 12 and flushed with the fluorescent substance 10 which remained on the plate 3 of glass as shown in drawing 5 (b), the sample DNA 11 which has not been combined with probe DNA 1 will be discharged. Then, as shown in drawing 5 (c), hybridization is detected by detecting the light which the fluorescent substance which is carrying out the indicator to the sample DNA combined with probe DNA is excited by the light energy from a lamp 14, and a fluorescent substance excites, and emits light by the photosensors 13, such as CCD.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention] According to this invention, the biochip which made the binder adhere only to the portion which carries out the spot of the probe can be manufactured, and detection sensitivity can be raised at the time of BA[CH[PPU read.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] In the experiment using BAICHIPPU, sprinkled Sample DNA over BAICHIPPU, and it was made to hybridize with the probe DNA which has carried out the spot to BAICHIPPU, and has detected which probe DNA and Sample DNA joined together. As pretreatment of detection, after hybridizing, in order to eliminate the sample DNA which was not combined, the biochip is washed with water. However, since the plate is completely coated with the binder of DNA and glass, the sample DNA which was not combined with probe DNA sticks to portions other than the need, i.e., the binder portion to which the spot of the probe DNA is not carried out, and the sample DNA 11 combined with the binder 4 is not removed from on the plate 3 of glass by washing in cold water, either. It became a noise at the time of detection, and appeared, and sensitivity was low. That is, since it remained as garbage on a biochip not in the unique combination with probe DNA but in the state where it only stuck to the binder 4, the fluorescent substance by which the indicator is carried out to the sample DNA was also excited and a part of sample DNA emitted light, it was detected as a noise and there was a problem that S/N became bad.
[0007] this invention was made in view of the trouble of such conventional technology, and aims at offering the biochip in which Sample DNA does not adhere to the portion except having plotted the probe of a plate, and offering the manufacture method of the biochip.

MEANS

[Means for Solving the Problem] In order to attain the aforementioned purpose, the binder of a probe and glass is made to adhere only to the particular part on the plate which carries out the spot of the probe in this invention. That is, in order not to make the binder of a probe and glass adhere in addition to the portion which carried out the spot of the probe to the plate, after making Sample DNA put in and hybridize, since the sample DNA which was not combined with a probe will disappear from on a chip if it washes away with water, the noise of it can be lost at the time of detection, it can raise S/N's, and serves as high sensitivity.
[0009] That is, in the biochip by which the biochip by this invention planted the probe in two or more positions on a plate using the binder which combines a plate and a probe, it is characterized by carrying out localization of the binder to the position in which the probe is planted.
[0010] The manufacture method of the biochip by this invention is characterized by planting in a plate the mixture which mixed the probe and the binder in the manufacture method of the biochip which plants a probe in a plate using the binder which combines this plate and a probe, and manufactures a biochip.
[0011] The manufacture method of the biochip by this invention is characterized by including the step which makes the binder of a probe and a plate adhere to the position on the plate in which a probe should be planted locally, and the step which plants a probe in the position on the plate to which the binder has adhered in the manufacture method of the biochip which plants a probe in a plate and manufactures a biochip again. A plate can be made glass. Moreover, it is desirable to plant a probe using the pin by which the pin head became depressed.
[0012] The pin of this invention is characterized by the pin head to which a probe is made to adhere having become depressed in the pin used for planting a probe in a plate. The pin of this invention is characterized by forming a cross-joint type slot in the pin head to which a probe is made to adhere in the pin used for planting a probe in a plate again.
[0013]
[Embodiments of the Invention] Hereafter, the operation gestalt of this invention is explained. Here, the case where DNA is used as a probe is explained. However, only DNA can be used as a probe and it can also use RNA or protein as a probe. Moreover, although the example using the glass plate as a plate explains, the membrane made of nylon etc. is usable in addition to glass.
[0014] Drawing 1 is drawing explaining the principle of the gestalt of operation of the 1st of this invention. As shown in drawing 1 (a), two or more kinds of probe DNA 1 is contained in the microplate 2. As shown in drawing 1 (b), the plate 3 of glass is used as a plate of a biochip. As shown in drawing 1 (c), the binder 4 of DNA and glass is poured distributively to each well of a microplate, and it mixes with probe DNA 1. As a binder 4 of DNA and glass, poly-l-Lysine or a carbodiimide can be used, for example.
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[0016] Drawing 2 is drawing explaining the principle of the gestalt of operation of the 2nd of this invention. As shown in drawing 2 (a), probe DNA 1 is contained in the microplate 2. As shown in drawing 2 (b), the glass plate 3 is used as a plate of BAICHIPPU. As shown in drawing 2 (c), a binder 4 is sucked up with a vas capillare 6, and it breathes out in the position which carries out the spot of the probe DNA on the glass plate 3. The spot of the binder 4 is carried out to the position which carries out the spot of the probe DNA 1 on

the glass plate 3, and it is made to adhere to it beforehand by carrying out like this. then, the probe DNA 1 which is contained in the microplate 2 as shown in drawing 2 (d) is sucked up by the pin 5 on the plate 3 formatted by the binder 4 (or a solution is adhered to a pin 5 — making), and a repeat spot is carried out In this way, as shown in drawing 2 (e), BAICHIPPU 20 in which a binder 4 adheres only to a required portion and the binder 4 has not adhered to the portion except having carried out the spot of the probe can be made.
[0017] The head configuration of the pin 5 used for drawing 6 by this invention is shown. Pin 5a shown in drawing 6 (a) is the pin by which the head portion, i.e., the portion to which a probe is made to adhere, became depressed in the arch type. Moreover, pin 5b shown in drawing 6 (b) is the pin which the head portion became depressed in the arch type, and cut the slot to the cross-joint type in the hollow further. By hollowing the head of a pin in the shape of an arch, when a pin is put in into the probe which carries out a spot, it is the structure from which a probe solution is well sucked in by the pin head with surface tension. The depth of an arch is arbitrary. By using this pin, the amount of DNA spots to a plate can be increased about 10 times from the case where the conventional pin with a flat head is used. Pin 5c shown in drawing 6 (c) cuts a slot to a cross-joint type on the front face of a flat head. A head can increase the amount of spots also by this pin as compared with the only flat conventional pin.
[0018] Drawing 3 is drawing explaining the principle of the hybridization using BAICHIPPU by this invention. Probe DNA 1 makes both hybridization solutions put in and hybridize the sample DNA 11 which carried out the indicator to BAICHIPPU 20 by which the spot is carried out to the glass plate 3 with the fluorescent substance 10 by the binder 4, as shown in drawing 3 (a). A hybridization solution is mixed liquor which consists of formaldehyde, SSC (NaCl, trisodium citrate) and SDS (sodium dodecyl sulfate), EDTA (ethylenediamidetetraacetic acid), distilled water, etc., and a mixed ratio changes with properties of DNA to be used.
[0019] If the probe DNA 1 on a sample DNA 11 and BAICHIPPU is a complementary strand DNA at this time, both will take a double helix structure and will join together. On the other hand, if both are not complementary strands, it will not join together, but the sample DNA 11 which carried out the indicator with the fluorescent substance 10 remains as garbage on the plate 3 of glass. If the sample DNA 11 which carried out the indicator is put in into water 12 and flushed with the fluorescent substance 10 which remained on the plate 3 of glass as shown in drawing 3 (b), since combination is weak, the sample DNA 11 of garbage will be discharged and glass and DNA will disappear from on the plate 3 of glass. As shown in drawing 3 (c), detection of hybridization excites the fluorescent substance which is carrying out the indicator to the united sample DNA with the light of a lamp 14, and detects the light in which a fluorescent substance excites and emits light by the photosensors 13, such as CCD. In order that there may be no sample DNA of garbage on a biochip 20 at this time, the S/N ratio of detection improves.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing explaining an example of the manufacture method of the biochip by this invention.
[Drawing 2] Drawing explaining other examples of the manufacture method of the biochip by this invention.
[Drawing 3] Explanatory drawing of the hybridization using BAICHIPPU of this invention, and detection.
[Drawing 4] Drawing explaining the manufacture method of the conventional biochip.
[Drawing 5] Explanatory drawing of the hybridization using conventional BAICHIPPU, and detection.
[Drawing 6] Explanatory drawing of the pin for probe adhesion by this invention.
[Description of Notations]
1 [— The plate of glass, 4 / — A binder, 5 / — A pin, 6 / — A vas capillare, 10 / — A fluorescent substance, 11 / — Sample DNA, 12 / — Water, 13 / — A photosensor, 14 / — A lamp, 20 / — Biochip.] — Probe DNA, 2 — The microplate for probe storing, 3

[Translation done.]

CORRECTION or AMENDMENT

[Official Gazette Type] Printing of amendment by the convention of 2 of Article 17 of patent law.
[Section partition] The 1st partition of the 1st section.
[Date of issue] December 11, Heisei 13 (2001. 12.11)

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C12N 15/09
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1/68
H01L 49/00

[FI]

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C12Q 1/00 Z
1/68 A
H01L 49/00 Z

[Procedure revision]
[Filing Date] June 1, Heisei 13 (2001. 6.1)
[Procedure amendment 1]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0002.
[Method of Amendment] Change.
[Proposed Amendment]
[0002]
[Description of the Prior Art] Carrying out the spot of the probe which consists of biopolymers, such as two or more kinds of DNA, RNA, and protein, to plates, such as glass, and manufacturing a biochip from the former, was performed. Drawing 4 is drawing explaining the principle of this conventional method. As shown in drawing 4 (a), the microplate 2 containing two or more kinds of probe DNA 1 is prepared. On the other hand, as shown in drawing 4 (b), the glass plate is prepared as a plate 3, and as drawing 4 (c) shows, the front face of a plate 3 is coated with poly-l-Lysine as a binder 4 of DNA and glass. Then, as drawing 4 (d) shows, the probe DNA 1 included in the microplate 2 is made to adhere to a pin, DNA and the probe DNA 1 made to adhere to a pin 5 on the glass plate 3 with which the binder (poly-l-Lysine) 4 of glass is coated are contacted on a front face, and a spot is carried out to it. This work was repeated until it finished carrying out the spot of all the probe DNA included in the microplate 2, and the biochip shown in drawing 4 (e) was manufactured. Thus, conventionally, the plate was beforehand coated with the binder of DNA and glass completely, on it, DNA was plotted and the biochip was manufactured.
[Procedure amendment 2]
[Document to be Amended] Specification.

[Item(s) to be Amended] 0003.
[Method of Amendment] Change.
[Proposed Amendment]
[0003] Drawing 5 is drawing explaining the principle of the hybridization using the biochip. Probe DNA 1 makes both hybridization solutions put in and hybridize the sample DNA 11 which carried out the indicator to the biochip by which the spot is carried out to the plate 3 of glass with the fluorescent substance 10 by the binder 4, as shown in drawing 5 (a). A hybridization solution is mixed liquor which consists of formaldehyde, SSC (NaCl, trisodiumcitrate) and SDS (sodium dodecyl sulfate), EDTA (ethylenediamidetetraacetic acid), distilled water, etc., and a mixed ratio changes with properties of DNA to be used.
[Procedure amendment 3]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0004.
[Method of Amendment] Change.
[Proposed Amendment]
[0004] If the probe DNA 1 on a sample DNA 11 and a biochip is a complementary strand DNA at this time, both will take a double helix structure and will join together. On the other hand, if both are not complementary strands, it will not join together, but the sample DNA 11 which carried out the indicator with the fluorescent substance 10 combines with the binder 4 coated on the plate 3 of glass, and remains as garbage.
[Procedure amendment 4]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0006.
[Method of Amendment] Change.
[Proposed Amendment]
[0006]
[Problem(s) to be Solved by the Invention] In the experiment using the biochip, sprinkled Sample DNA over the biochip, and it was made to hybridize with the probe DNA which has carried out the spot to the biochip, and has detected which probe DNA and Sample DNA joined together. As pretreatment of detection, after hybridizing, in order to eliminate the sample DNA which was not combined, the biochip is washed with water. However, since the plate is completely coated with the binder of DNA and glass, the sample DNA which was not combined with probe DNA sticks to portions other than the need, i.e., the binder portion to which the spot of the probe DNA is not carried out, and the sample DNA 11 combined with the binder 4 is not removed from on the plate 3 of glass by washing in cold water, either. It became a noise at the time of detection, and appeared, and sensitivity was low. That is, since it remained as garbage on a biochip not in the unique combination with probe DNA but in the state where it only stuck to the binder 4, the fluorescent substance by which the indicator is carried out to the sample DNA was also excited and a part of sample DNA emitted light, it was detected as a noise and there was a problem that S/N became bad.
[Procedure amendment 5]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0015.
[Method of Amendment] Change.
[Proposed Amendment]
[0015] next, what mixed probe DNA 1 with the binder 4 as shown in drawing 1 (d) is sucked up by the pin 5 (or a solution is adhered to the point of a pin 5 — making), and a spot is carried out to a plate 3 As shown in drawing 1 (e), the biochip 20 in which a binder 4 adheres only to a required portion and the binder 4 has not adhered to the portion except having carried out the spot of the probe can be made from performing this processing repeatedly to all the probe DNA included in the microplate 2.
[Procedure amendment 6]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0016.
[Method of Amendment] Change.
[Proposed Amendment]
[0016] Drawing 2 is drawing explaining the principle of the form of operation of the 2nd of this invention. As shown in drawing 2 (a), probe DNA 1 is contained in the microplate 2. As shown in drawing 2 (b), the glass plate 3 is used as a plate of a biochip. As shown in drawing 2 (c), a binder 4 is sucked up with a capillary tube

6, and it breathes out in the position which carries out the spot of the probe DNA on the glass plate 3. The spot of the binder 4 is carried out to the position which carries out the spot of the probe DNA 1 on the glass plate 3, and it is made to adhere to it beforehand by carrying out like this. then, the probe DNA 1 which is contained in the microplate 2 as shown in drawing 2 (d) is sucked up by the pin 5 on the plate 3 formatted by the binder 4 (or a solution is adhered to a pin 5 — making), and a repeat spot is carried out In this way, as shown in drawing 2 (e), the biochip 20 in which a binder 4 adheres only to a required portion and the binder 4 has not adhered to the portion except having carried out the spot of the probe can be made.

[Procedure amendment 7]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0018.
[Method of Amendment] Change.
[Proposed Amendment]
[0018] Drawing 3 is drawing explaining the principle of the hybridization using the biochip by this invention. Probe DNA 1 makes both hybridization solutions put in and hybridize the sample DNA 11 which carried out the indicator to the biochip 20 by which the spot is carried out to the glass plate 3 with the fluorescent substance 10 by the binder 4, as shown in drawing 3 (a). A hybridization solution is mixed liquor which consists of formaldehyde, SSC (NaCl, trisodium citrate) and SDS (sodium dodecyl sulfate), EDTA (ethylenediamidetetraacetic acid), distilled water, etc., and a mixed ratio changes with properties of DNA to be used.

[Procedure amendment 8]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0019.
[Method of Amendment] Change.
[Proposed Amendment]
[0019] If the probe DNA 1 on a sample DNA 11 and a biochip is a complementary strand DNA at this time, both will take a double helix structure and will join together. On the other hand, if both are not complementary strands, it will not join together, but the sample DNA 11 which carried out the indicator with the fluorescent substance 10 remains as garbage on the plate 3 of glass. If the sample DNA 11 which carried out the indicator is put in into water 12 and flushed with the fluorescent substance 10 which remained on the plate 3 of glass as shown in drawing 3 (b), since combination is weak, the sample DNA 11 of garbage will be discharged and glass and DNA will disappear from on the plate 3 of glass. As shown in drawing 3 (c), detection of hybridization excites the fluorescent substance which is carrying out the indicator to the united sample DNA with the light of a lamp 14, and detects the light in which a fluorescent substance excites and emits light by the photosensors 13, such as CCD. In order that there may be no sample DNA of garbage on a biochip 20 at this time, the S/N ratio of detection improves.

[Procedure amendment 9]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0020.
[Method of Amendment] Change.
[Proposed Amendment]
[0020]
[Effect of the Invention] According to this invention, the biochip which made the binder adhere only to the portion which carries out the spot of the probe can be manufactured, and detection sensitivity can be raised at the time of biochip read.

[Translation done.]